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Short Communication

A three-stage culture process for improved exopolysaccharide production by *Tremella fuciformis*

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ABSTRACT

Tremella fuciformis produces several bioactive secondary metabolites including exopolysaccharides. Cultivation of the fungus was carried out in a three-stage process consisting of a 1.5-day cultivation with orbital shaking at 200 rpm, a 1.5-day cultivation with reciprocal shaking at 200 strokes, and a 1.5-day cultivation with orbital shaking at 200 rpm. Exopolysaccharide production and specific production rate reached 5.80 g L^{-1} and 0.15 d^{-1}, respectively, which is an increase of 260% and 200% compared with the corresponding values for fermentations with orbital shaking only, and of 243% and 150% compared with the corresponding values for fermentations with reciprocal shaking only. The three-stage culture method is time-saving and easy to operate.

1. Introduction

Tremella fuciformis Berk, a traditional Chinese edible and medicinal mushroom, grows as a yeast-like monocyte that reproduces by budding. A single monocyte can rapidly form a colony and grow by vegetative propagation. *Tremella* polysaccharides have been reported to stimulate the immune system, have antitumor activity (Sun et al., 2009), inhibit quorum sensing by interacting with receptor proteins (Zhu and Sun, 2008), and exhibit hypoglycemic activity (Zhu et al., 2006). Commercially, polysaccharides from *T. fuciformis* are predominantly obtained through field-cultivation of the fruiting body, but this form of cultivation cannot control the quality of the final product. Submerged cultivation of *T. fuciformis* is therefore viewed as a promising alternative for efficient production of exopolysaccharide (EPS) on a large scale (Zhu and Sun, 2009). In submerged fermentation of mushrooms, batch and batch-fed fermentation processes are usually adopted to achieve a high yield of bioactive metabolites by improving cell density, but these methods are laborious, time-consuming and expensive. Furthermore, the full process from laboratory to scale-up pilot batch is not easily controlled. In the present study, a three-stage culture method was developed, which greatly improved dry cell weight (DCW) and EPS production in submerged fermentation of yeast-like spore. The process consisted of 1.5-day culture with orbital shaking, 1.5-day culture with reciprocal shaking, and 1.5-day cultivation with orbital shaking. This approach allowed simple control over dissolved oxygen (DO), carbon dioxide (CO_2), pH and foam formation. In the culture process, different shakers can provide different shear stresses and oxygen uptake, both of which affect the bioprocess.

2. Methods

2.1. Strain and media

T. fuciformis ACCC 50546 from the Agricultural Culture Collection of China (Beijing) was used for development and evaluation of the three-stage culture. The stock culture was maintained on potato dextrose agar (PDA) slants. After 10 days, yeast-like conidia were isolated from mycelia, incubated on PDA slant at 28 °C for 5 days, then stored at 8 °C and subcultured once a month.

2.2. Inoculum preparation

T. fuciformis spores from 5-day-old slant cultures were transferred to the seed culture medium with a sterilized inoculating loop. The seed culture was grown in a 250-ml flask containing 50 ml of fermentation medium (2.0% glucose, 0.2% yeast extract, 0.2% peptone, 0.05% $MgSO_4{\cdot}7H_2O$, 0.1% K_2HPO_4, 0.046% KH_2PO_4) at 28 °C on a rotary shaker incubator at 200 rpm for 3 days. The spore inoculum was used for further experiments.

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